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LEPTOTHRIX INNOMINATA (MILLER)*

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Leptothrix innominata, so commonly found in the "materia alba" along the human gingival margin, is apparently a strict parasite. That

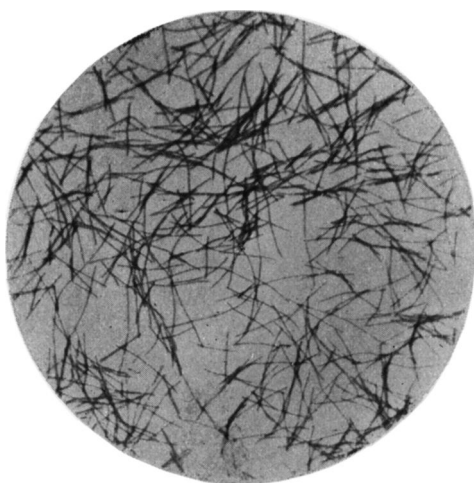


Fig. 1. *Leptothrix innominata* from anaerobic + 0.5 Martin's pleuritic slant. Methyl alcohol, dilute carbolfuchsin. \times 500.

is, its nourishment is largely dependent on the presence of certain proteins in the human body. According to Goadby¹ and Küster,² it has never been cultivated. We have isolated it in pure culture only once. This was due not to the difficulty of isolation so much as to the fact that at this time we became interested in other species. For the isolation of parasitic species we have been using Martin's medium³ with reactions varying from +0.5 to +2, the medium

containing an equal quantity of ascites or pleuritic fluid that had been heated to 56 C. for 1 hour on 5 or 6 successive days. Our work shows that this organism is adapted to a wide range of oxygen tension. It is a microaerophile-anaerobe; but we have not succeeded in obtaining an aerobic strain from it. This adaptation is what one might expect when one considers its natural habitat. When once grown away from the human tissues, it can thrive on beef-extract agar provided some suitable carbohydrate is present. Its marked action on the primary and secondary

* Received for publication February 24, 1916.

¹ The Mycology of the Mouth, 1903.

² Kolle and Wassermann's Handb. d. pathogen. Mikroorganismen, 1913, 6, p. 441.

³ Jour. Path. and Bacteriol., 1911, 15, p. 76.

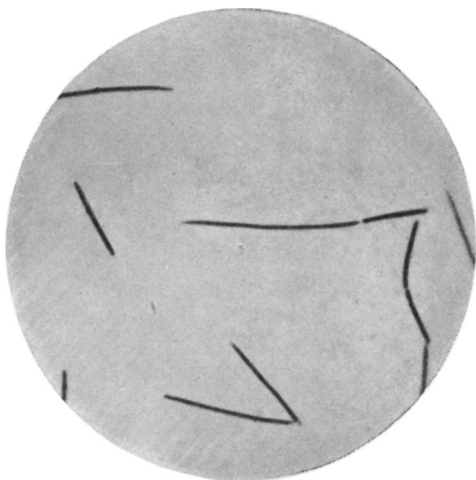


Fig. 2. Same as Fig. 1. $\times 1000$.

carbohydrates is noteworthy. Occasionally leptothrices which give the granulose reaction are encountered in the human mouth, tho the vast majority stain yellow with Gram's iodine solution. In the presence of maltose a few *Leptothrix innominata* also give the granulose reaction. This speaks for a unity of species.

MORPHOLOGY

The organism is rarely seen as a single cell but is commonly encountered in twos, and threes, and chains of 7 or more segments. Each segment is an elongated rod, usually with rounded ends tho frequently terminal segments may exhibit a tapering, somewhat pointed free end (Figs. 1, 2, and 3). When measured with a filar micrometer under the Zeiss 3 mm., N. A. 1.40, the cells in fresh unstained condition are 1.4 microns in diameter, but when fixed with methyl alcohol and stained with dilute carbolfuchsin, they are 0.84 micron in diameter. Single segments vary from 5 to 14 microns or more. Thus: 3 segments measured 26.5 microns, 7 segments measured 45.36 microns. The segments are usually attached to one another in a straight line and seem to be covered by a common delicate envelope. Long chains of segments—as in the milk culture, 336 microns long—bend like a hair and not like a chain of loosely joined rods.

The cytoplasm of the segments is fairly homogeneous but often shows a fine vacuolization along the central longitudinal axis. In cultures a week old on media containing fermentable carbohydrates, the cytoplasm is markedly vacuolated and the stained cells show many small irregularly distributed and deeply stained areas. No bodies resembling spores were seen. Nor were there any signs of motility in sealed and unsealed preparations.

In an anaerobic culture on +1 maltose agar, grown for 8 days at 37 C., a number of coiled forms were encountered. These apparently

coiled like a corkscrew at one end (Fig. 4) and then bent over and grew around the lengthening filament, thus resembling a twisted loop of rope (Fig. 5). These forms did not stain well and yet they appeared normal in the fresh preparations.

In scrapings from the teeth we have encountered forms showing a terminal pyriform swelling and such forms were found in pleuritic-fluid potato-water cultures, where also there were present fewer free ovoid and pyriform bodies (Fig. 6). These have about the same refractive index as the vegetative cells.

STAINING REACTIONS

Smears from cultures fixed by heat or methyl alcohol, stain readily with dilute carbolfuchsin or with Bordet's carbol toluidin blue or with Nicolle's carbol gentian violet. There is rather faint staining with Löffler's methylene blue in 5 minutes. The leptothrix loses the stain on treatment by Gram's method.

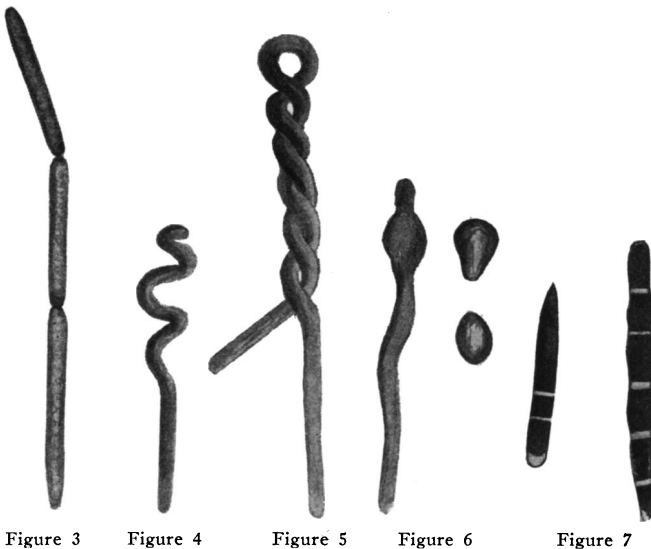


Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Fig. 3. Drawing of a fresh preparation from a colony on + 0.5 Martin's pleuritic medium, 48 hours at 37 C.

Fig. 4. Drawing of beginning looped form, from a fresh preparation from an 8-day + 1 maltose-agar anaerobic culture.

Fig. 5. Completion of looped form. Same source as Fig. 4.

Fig. 6. Swollen and pyriform and ovoid bodies from anaerobic pleuritic-fluid potato-water culture.

Fig. 7. Granulose reaction given by a few organisms in an anaerobic + 1 maltose-agar culture grown for 6 days at 37 C.

Granulose Reaction.—Most of the cultures were mounted in Gram's iodine solution and throughout the organisms were stained yellow except those from the +1 maltose anaerobic culture kept for 6 days at 37 C. Here a few, probably less than 1%, gave a marked reaction, the granulose being present in large blocks with intervening colorless segments (Fig. 7).

Isolation and Biochemical Characters.—Scrapings from the line of the human gum were suspended in sterile 0.85% salt solution, centrifugated, and resuspended 3 times. In this way a large number of the smaller associated bacteria were removed. The sediment was then spread on the surface of the medium, which had been solidified in Petri dishes. Various modifications of Martin's medium were used but the best result was obtained with the following: Martin's medium containing 1% of sodium sulfite and almost an equal volume of sterile human pleuritic fluid (heated to 56 C. for 5 successive days). The plates were kept at 37 C. under anaerobic conditions by means of pyrogallie acid and caustic soda, according to the plan suggested by Lentz.⁴ The colonies first appeared as a loose thatch-like mass, which grew and became visible to the naked eye in from 4 to 5 days. When transplanted to slants of the same medium, the growth was much more luxuriant, isolated colonies reaching a diameter of from 0.5 to 3 mm. in a few days. Isolated colonies presented a fairly characteristic appearance when viewed through a hand lens; that is, they appeared like Medusa-lock colonies and as the irregularities in the thickness of different wavy portions of the colonies refracted the light, a pearly iridescence was exhibited (Fig. 8). This kind of colony, however, is not peculiar to the leptothrix, but may be shown by other bacteria which form threads; for example, *B. coli* and *B. typhosus* grown under partial tension.

Transplants to plain Martin's pleuritic medium grew equally well under anaerobic conditions and under partial oxygen tension produced by means of *B. subtilis*. Subcultures from the anaerobic and partial-tension cultures to aerobic slants of the same medium repeatedly remained sterile. The organism was planted on the following media and kept under anaerobic conditions with results as indicated. Growth fairly luxuriant on +1 maltose agar, Löffler's blood serum, +1 glucose agar containing blood—where the medium as well as the growth turned to a brownish-red hue—and on +1 lactose agar. No growth on +1 agar, +1 dextrose agar, +1 glycerin agar, +1 broth, +1 dextrose broth, +1 Dunham's peptone solution made from Liebig's extract. In litmus milk growth took place with the production of acid without coagulation, and the formation of a yellowish waxy mass which floated on the sur-

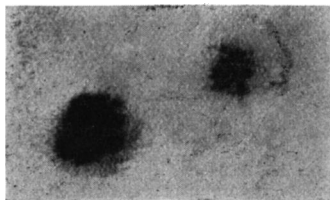


Fig. 8. Colonies of *Leptothrix innominata*. Four days' growth at 37 C., anaerobic, in + 0.5 Martin's pleuritic medium. $\times 6$.

face and resembled cerumen from the external ear. Microscopically this was composed entirely of coarse and fine fatty-acid crystals. The leptothrices were found only in the sediment, where they presented the usual appearance and breadth but many filaments—one, 336 microns long, was measured with the filar micrometer. These filaments contained irregularly placed spherical refractile bodies of the same diameter as the filaments themselves. They did not give the granulose reaction.

There was no growth on plain potato, but in a potato culture to which pleuritic fluid had been added, a flocculent growth appeared in the surrounding fluid. This was composed of normal forms, but many filaments showed irregular globular swellings and some free ovoid globular forms 3.3 microns in diameter. None of these forms gave the granulose reaction (Fig. 6).

Fermentation Reactions.—These tests were made according to the method recommended by Martin;³ that is, to +0.5 Martin's medium in tubes, there were added a few drops of a 10% carbohydrate or alcohol solution in distilled water (previously sterilized at 15 pounds for 15 minutes), and sufficient sterile litmus and pleuritic fluid. On such anaerobic slants there was marked acid-production with precipitation of serum in dextrose, levulose, galactose, lactose, saccharose, maltose, inulin, dextrin, raffinose, and mannose; mannite, erythrite, dulcitate, and isodulcitate were not attacked.

³ Centralbl. f. Bakteriöl., I, O., 1910, 53, p. 358.